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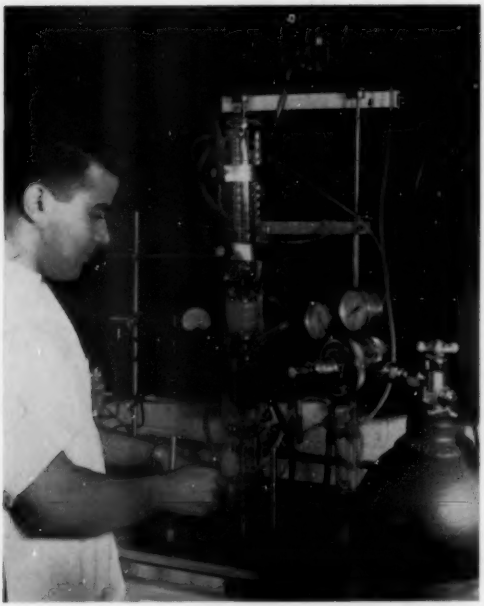

Heart Perfusion Apparatus in LaWall Memorial Laboratory of Pharmacology and Biochemistry

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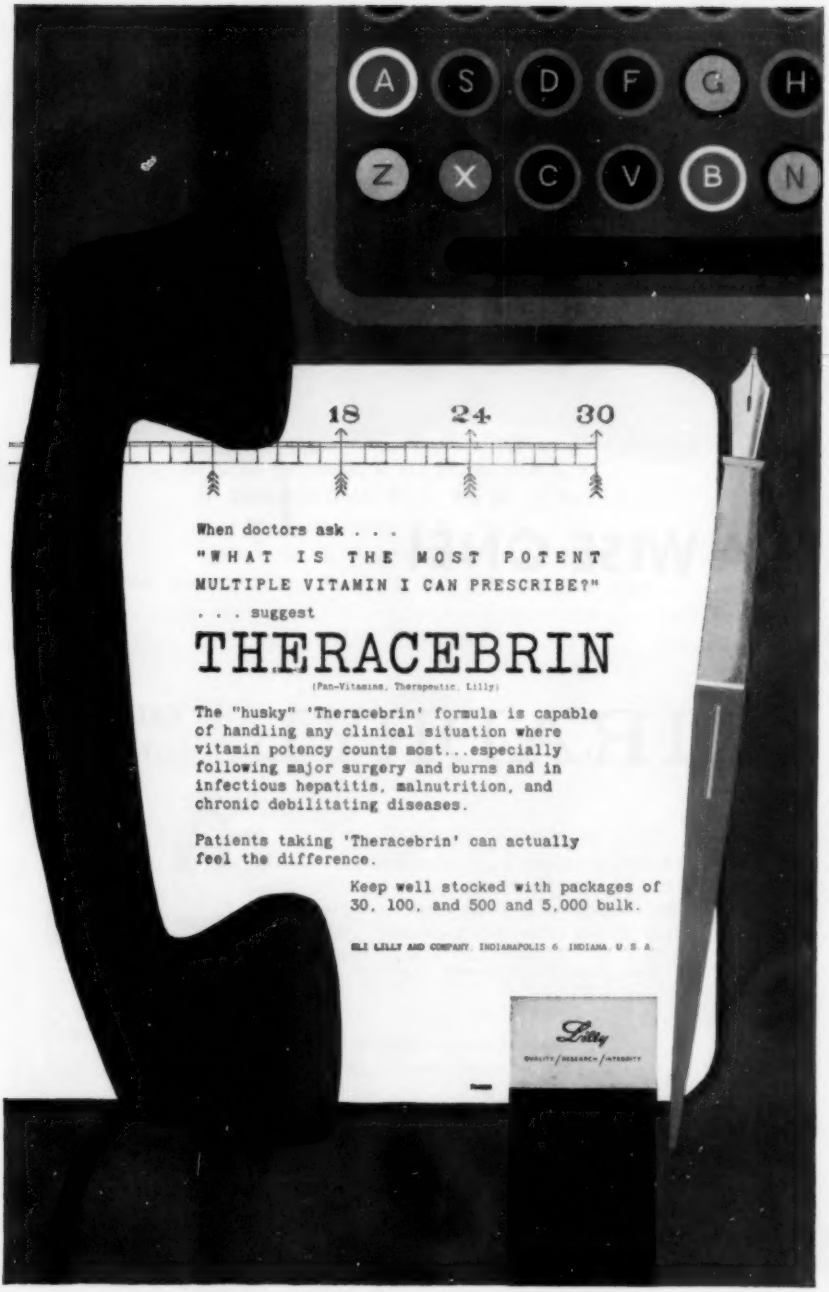
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AMERICAN JOURNAL OF PHARMACY

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Since 1825

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CONTENTS

E D I T O R I A L

ON WHOSE TEAM ARE WE?

NOTHING has contributed more to the resurgence of professionalism in pharmacy or to its improved economic status than has the amazing development of modern medicinals in the last two decades. As every successful pharmacist knows, the most profitable department in the pharmacy is the prescription department and, while pharmacists grouse about high inventories and the cost of doing business, the truth is that in recent years the prescription pharmacy has done quite well. An analysis of typical drugstore operations leads one to the conclusion that the success or failure of the modern pharmacy depends almost entirely on whether or not prescription volume can be built and maintained.

We hear today in many pharmaceutical circles an expression which seems to be catching on and even accepted by our medical colleagues. This expression is that pharmacy, more than ever before, is a part of the public health team. That it must be an integral part of this team and continue to grow in this direction should be crystal clear to all thinking persons as the trend concerning pharmacy and its relationship to medicine is studied. Either pharmacy will continue to fortify its present position or it may cease to be a profession.

With our future so clearly defined for us, it is somewhat ironic that so many pharmacies still lend themselves to the quackery and empiricism of that by-gone age when medicine men and carnival barkers promoted sure cures for every human ailment. It must be both painful and perplexing for the practicing physician who really wants to accept the pharmacist as a full-fledged professional colleague to see this same pharmacist give respectability to products which every well-informed person knows are absolutely worthless and nothing more than blatant efforts to capitalize on the ignorant layman's belief that he can diagnose and treat his own ailments. The window streamers, show cards, displays, and even classified advertisements in many newspapers tell the story of these nostrums known only too well since they have disgraced pharmacy for years. While some

of these products eventually die belated deaths, others just as unscientific and disgraceful manage to take their place.

Just the other day, we saw advertised by a community pharmacist a famous old-time "kidney" pill. The word kidney has long since been deleted from the name under government pressure. Just why similar pressure has not been exerted to ban the product from the market is not clear. The list of ingredients shows that these pills contain not a single ingredient that might prove helpful to the kidneys and, in the accompanying literature, the symptoms described as stemming from improper kidney function are utterly ridiculous. This is but one single example of dozens of products on the shelves of many pharmacies and yet not a single person, from the manufacturer to the pharmacist, has the slightest interest in the welfare of those to whom such products are sold. No pharmacist in his right mind would take such a product himself or recommend them to someone in his immediate family; yet, he continues to sell such products to the public and even lends encouragement to such sales.

It is small wonder that pharmacy "trips over its own skirts" in its faltering climb up the stairway of professionalism. It is also small wonder that we are at times not listened to very attentively in our efforts to promote interprofessional relations, gain better recognition, seek protective legislation, and do all of the other things which prove to be so difficult.

It is high time that organized pharmacy takes the necessary steps to establish clearly on which team we propose to play. Shall it be the bona fide public health team which needs us and welcomes us, or shall it be a team made of of fakers, quacks, and charlatans? No one to our knowledge has managed to play on two opposing teams and do it successfully at the same time.

L. F. Tice

AN EVALUATION OF THE ANTICONVULSANT ACTIVITY OF A NEW BARBITURIC ACID DERIVATIVE

5-(1-methylbutyl)-5-propargyl barbituric acid [1]

By G. Victor Rossi and Hernando Arciniegas

IT is generally recognized that, in anesthetic doses, all of the clinically employed barbiturates are capable of inhibiting convulsions of various origins (1). However, relatively few barbituric acid derivatives (notably Phenobarbital, Mephobarbital and Metharbital) manifest selective anticonvulsant activity in doses which cause only mild sedation.

On the basis of anticonvulsant potency and favorable therapeutic index, as determined by preliminary screening procedures, the compound 5-(1-methylbutyl)-5-propargyl barbituric acid (A-2367) [2] was selected for further study from a series of newly synthesized propargyl barbiturates. This report is concerned with an evaluation of the anticonvulsant activity of compound A-2367 against both chemically (Metrazol) and electrically induced seizures in mice and rats.

Experimental

Female Swiss Webster mice (Taconic Farms) weighing from 18 to 22 Gm. and female Wistar rats (Dierolf Farms) weighing from 100 to 120 Gm., maintained in temperature and humidity controlled quarters, were used throughout the experimental procedures. All animals were permitted free access to Rockland Rat Diet and tap water except during the actual test period. In all of the following procedures the anticonvulsants examined were injected intraperitoneally in a solvent consisting of 25% propylene glycol in distilled water. The amounts of propylene glycol administered do not alter the thresholds or patterns of experimental seizures (2).

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1. Received from the LaWall Memorial Laboratory of Pharmacology & Biochemistry, Philadelphia College of Pharmacy and Science, Philadelphia 4, Pennsylvania.

2. National Drug Company Research Designation.

This study was supported in part by a research grant from the National Drug Company, Philadelphia 44, Pennsylvania.

TABLE 1

HYPNOTIC EFFECT OF A-2367 IN MICE

Group Number*	A-2367 mg./Kg.	Per cent Response	Mean Sleeping Time**
1	35	55	58
2	40	90	98 ± 15
3	45	100	167 ± 18
4	50	100	190 ± 23
5	55	100	269 ± 28
6	60	100	258 ± 28
7	65	100	268 ± 24

* N = 20 in all groups.

** Mean sleeping time and standard error reported in minutes.

TABLE 2

ANTICONVULSANT ACTIVITY OF A-2367 IN MICE METRAZOL TEST *

Group Number	A-2367 mg./Kg.	Trimethadione mg./Kg.	Per cent Protection	No. Deaths No. Mice
1	—	—	0	15/20
2	10	—	10	6/20
3	20	—	90	0/20
4	30	—	80	0/20
5	40	—	100	0/20
6	—	500	90	0/20

* Metrazol Dose: 85 mg./Kg., s.c.

Determination of Hypnotic Activity.—Seven groups each composed of 20 mice received intraperitoneal injections of compound A-2367 in doses varying by 5 mg. increments from 35 to 65 mg./Kg. The interval between administration of the barbiturate and reappearance of the "righting reflex" (animal turns spontaneously over into normal position but continues to sleep) was recorded as the "sleeping time" (3).

Determination of Anticonvulsant Activity.—Compound A-2367 was compared with Trimethadione for its ability to modify chemically (Metrazol) induced convulsions in mice, and with Diphenylhydantoin Sodium and Phenobarbital Sodium for its activity against electrically induced convulsions in mice and rats.

The maximal Metrazol seizure (M. M. S.) test measures the ability of anticonvulsants to afford complete protection against seizures induced by subcutaneous injection of a convulsant dose (CD_{97}) of Metrazol (85 mg./Kg.) in mice (2). Details of the assay have been described by Swinyard and his associates (2, 4). Four groups of 20 mice each were injected with 10, 20, 30, and 40 mg./Kg. of A-2367, respectively. A control group received only injections of the solvent. A sixth group was given an anticonvulsant dose of 500 mg./Kg. of Trimethadione (2), a dose which has little effect on normal activity and represents approximately one-fifth of the LD_{50} (5). Thirty minutes after administration of the anticonvulsant agents, the animals were injected with the convulsant dose of Metrazol and observed continually during the succeeding two hour period for the appearance of threshold seizures (4).

The maximal electroshock seizure (M. E. S.) test measures the ability of anticonvulsant drugs to abolish the hindlimb tonic extensor component of the maximal seizure pattern induced by 50 mA of current in mice or 150 mA in rats delivered for 0.2 second (2). Current was conducted from the Stimulator [3] by corneal electrodes placed on the eyeballs previously moistened with a drop of 1.0% Butacaine Sulfate solution.

The degree and duration of protection against maximal electroshock seizures was determined for 20, 40, 50 and 60 mg./Kg. doses of compound A-2367 in mice, and compared with the protection provided by 10 and 20 mg./Kg. doses of Diphenylhydantoin Sodium

3. Electroshock Seizure Apparatus, Model 2-C, Hans Technical Associates, Palo Alto, California.

TABLE 3

ANTICONVULSANT ACTIVITY OF A-2367 IN MICE M. E. S. TEST *

Group** Number	A-2367 mg./Kg.	Per cent Protection Hours After Administration of Anticonvulsant ½	1	2	4	6	8	10
1	—	0						
2	—		0					
3	—			0				
4	—				0			
5	—					0		
6	—						0	
7	—							0
8	20	75						
9	20		40					
10	20			10				
11	40	100						
12	40		100					
13	40			100				
14	40				45			
15	40					10		
16	40						0	
17	50			100				
18	50				90			
19	50					70		
20	50						5	
21	60				100			
22	60					100		
23	60						55	
24	60							0

* M. E. S. = Maximal Electroshock Seizure Test. Current of 50mA delivered through eyeball electrodes for duration of 0.2 second.

** N = 20 in all groups.

and 20 and 30 mg./Kg. doses of Phenobarbital Sodium. Different groups, each consisting of 20 mice, were used for each M. E. S. test at intervals of ½, 1, 2, 4, 6, 8 and occasionally 10 hours after administration of the anticonvulsants. This experimental design avoids modification of seizure patterns resulting from too frequent repetition of the test on the same animal (6). At each designated time interval maximal electroshock seizures were elicited in separate groups of 20 control mice which had received only intraperitoneal injections of the solvent.

A series of experiments was also conducted to compare the anticonvulsant activity of 20 mg./Kg. of compound A-2367 with 10 mg./Kg. of Diphenylhydantoin Sodium by the M. E. S. test in rats.

Results and Discussion

Hypnotic Activity.—At the dose levels of compound A-2367 employed, an initial stage of stimulation characterized by increased motor activity and hyperpnea was observed before the onset of hypnosis. This primary excitation resembled that typically elicited in mice by many barbiturates. The percentage of animals manifesting disappearance of the "righting reflex" and the average duration of the "sleeping time" at each dose level are given in Table 1. It may be noted that the mean duration of hypnotic effect increased progressively with increasing doses up to 55 mg./Kg. of compound A-2367, at which concentration a plateau was attained; further increments of drug did not produce additional increase in "sleeping time". The ED_{100} of compound A-2367 which produced a mean "sleeping time" of about 2 hours (45 mg./Kg.) represents approximately ½ of the intraperitoneal LD_{50} (90.4 mg./Kg. with 95% Fiducial Limits of 98.0 and 84.0 mg./Kg.).

Anticonvulsant Activity.—The degree of protection against the convulsive and the lethal effects of Metrazol in mice provided by compound A-2367 and Trimethadione are summarized in Table 2. All animals receiving only solvent and Metrazol CD_{97} (85 mg./Kg., subcutaneously) exhibited typical repetitive episodes of clonic spasms. In this control group, 75% mortality was recorded. High degrees of protection were obtained with 20 and 30 mg./Kg. doses of A-2367, while 40 mg./Kg. of the propargyl barbiturate completely abolished threshold seizures. In contrast, 500 mg./Kg. of Trimethadione was required to provide a comparable degree of anticonvulsant activity.

TABLE 4

ANTICONVULSANT ACTIVITY OF DIPHENYLHYDANTOIN SODIUM AND PHENOBABITAL SODIUM IN MICE M. E. S. TEST *

Group** Number	Diphenylhydantoin Sodium mg./Kg.	Per cent Protection Hours After Administration of Anticonvulsant ½	1	2	4	6	8
1	10	30					
2	10		40				
3	10			70			
4	10				45		
5	10					10	
6	20	100					
7	20		100				
8	20			100			
9	20				95		
10	20					80	
11	20						40
	Phenobarbital Sodium mg./Kg.						
12	20	30					
13	20		50				
14	20			60			
15	20				40		
16	20					15	
17	30	30					
18	30		90				
19	30			80			
20	30				60		
21	30					30	
22	30						30

* See legend to Table 3.
** N = 20 in all groups.

It should be emphasized, however, that the minimal effective anticonvulsant dose of 20 mg./Kg. of A-2367 produced a certain degree of ataxia and general depression in the mice, whereas Trimethadione, in the dose employed, did not alter the normal activity of the animals.

The relative degree and duration of protection offered against maximal electroshock seizures in mice by varying doses of compound A-2367 are given in Table 3. Comparable data obtained with Diphenylhydantoin Sodium and Phenobarbital Sodium are presented in Table 4. All control animals (Groups 1 to 7, Table 3) exhibited a definite extension of the hindlimbs during the tonic phase of the seizure. A dose of 20 mg./Kg. of A-2367 provided only partial anticonvulsant protection, whereas 40 mg./Kg. completely abolished the hindlimb tonic extensor component of the maximal seizure pattern. Further increases in drug concentration to 60 mg./Kg. progressively extended the duration of significant anticonvulsant activity. Examination of the data presented in Tables 2 and 3 indicates that compound A-2367 is considerably more potent on the basis of the M. M. S. test than it is by the M. E. S. test. Again, it is clearly evident by comparison of Tables 1 and 3, that doses of the propargyl barbiturate effective against maximal electroshock seizures in mice fall within the range which demonstrated considerable hypnotic activity.

Considering both the degree and duration of anticonvulsant effectiveness, compound A-2367 appears to be slightly more than half as potent as Phenobarbital Sodium, and somewhat less than half as active as Diphenylhydantoin Sodium on the basis of the M. E. S. response in mice. Diphenylhydantoin Sodium, which is characterized by its ability to modify the pattern of tonic-clonic seizures induced by maximal electroshock (7), did not alter the general level of activity of the animals in the doses employed. However, Phenobarbital Sodium in doses of 20 and 30 mg./Kg. produced ataxia and marked sedation.

Results of the M. E. S. test obtained with 20 mg./Kg. of compound A-2367 and 10 mg./Kg. of Diphenylhydantoin Sodium in young adult rats are summarized in Table 5. Equivalent protection in the mouse required 40 mg./Kg. of compound A-2367, indicating that this barbiturate is approximately twice as potent in rats as in mice on the basis of the M. E. S. test. A similar activity relationship between the two species has been reported to exist with Phenobarbital Sodium (2). Mild depression resulted with the dose of A-2367 used, whereas

the dose of Diphenylhydantoin Sodium administered did not produce detectable change in activity.

The value of employing a battery of experimentally induced seizures for the laboratory evaluation of potential anticonvulsant drugs has been repeatedly documented, since not all of the therapeutically effective agents exhibit activity by all of the standard procedures (2, 4, 7). However, the barbiturates proven to be clinically valuable in the treatment of epilepsy show activity by all of the four routinely used assay methods (4). This study therefore chose to determine the ability of a new barbiturate (compound A-2367) to modify representative chemically (Metrazol) and electrically induced seizures.

TABLE 5

ANTICONVULSANT ACTIVITY OF A-2367 AND DIPHENYLHYDANTOIN SODIUM IN RATS M. E. S. TEST *

		Per cent Protection					
Group**	A-2367	Hours After Administration of Anticonvulsant					
Number	mg./Kg.	½	1	2	4	6	9
1	20	100					
2	20		100				
3	20			100			
4	20				40		
5	20					40	
6	20						30
	Diphenyl-hydantoin Sodium mg./Kg.						
7	10	100					
8	10		40				
9	10			10			

* Current of 150 mA delivered through eyeball electrodes for duration of 0.2 second.

** N = 20 in all groups.

Summary

The data presented in this paper indicate that 5-(1-methylbutyl)-5-propargyl barbituric acid (A-2367) possesses marked anticonvulsant activity in mice and rats as determined by the maximal Metrazol seizure (M. M. S.) and maximal electroshock seizure (M. E. S.) tests. Compound A-2367 proved to be considerably more potent than Trimethadone against Metrazol induced convulsions in mice, but significantly less effective than Diphenylhydantoin Sodium and Phenobarbital Sodium in protecting mice against maximal electroshock seizures. Anticonvulsant doses of A-2367 generally fall within the range producing sedation and hypnosis, whereas equipotent concentrations of Trimethadione (M. M. S.) test and Diphenylhydantoin Sodium (M. E. S.) test do not alter normal activity. Phenobarbital Sodium in doses offering equivalent protection frequently causes quiescence but not hypnosis.

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The authors wish to acknowledge the assistance of Thomas H. F. Smith.

THE EFFECT OF pH ON THE STABILITY OF SOLUTIONS OF LIVER FRACTION I, N. F. X IN THE PRESENCE OF FERROUS LACTATE AND FERROUS GLUCONATE *

By Homi H. Kavarana,[1] Frederick V. Lofgren,[2] and Henry M. Burlage [3]

A Liver Fraction I sample was assayed for nicotinic acid, riboflavin and choline and found to meet N. F. sepcifications. Three series of nine buffered solutions each ranging from pH 3.0 to 7.0 were made using the above sample. The first series contained no addition of iron compound, the second series contained added ferrous gluconate U. S. P., the third series contained added ferrous lactate.

All these solutions were assayed as soon as prepared and after an aging period. The results of the assays of the aged solutions were compared with the original assays to determine the effect of pH and the presence of iron compounds on the stability of Liver Fraction I.

LIVER Fraction I and a number of other liver products are still recognized in The National Formulary (1) for use in some non-pernicious anemias and as a source of some of the B-complex vitamins. Liver Fraction I is generally presented to the public in the form of liquid preparations, designated as hematinics, usually fortified by the addition of vitamins and minerals, especially iron. Wilken, Lofgren and Burlage (2) reported on the stability of Liver Fraction I N. F. IX in buffered solutions ranging from pH 3.0 to 7.0 without added iron, with added ferrous sulfate U. S. P. and with added ferric ammonium citrate U. S. P.

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* Presented to the Section on Practical Pharmacy, A. Ph. A., New York Meeting, May, 1957.

1. Abstracted from a thesis submitted by Homi H. Kavarana to the Graduate School, University of Texas, in partial fulfillment of the requirements for the degree of Master of Science in Pharmacy.

2. Associate Professor of Pharmacy, College of Pharmacy, University of Texas, Austin.

3. Dean, College of Pharmacy, University of Texas, Austin.

The purpose of this study is to continue the previous study by reporting on the stability of Liver Fraction I using the same type of buffered solutions but adding iron in one series as ferrous gluconate U. S. P. and in another series with ferrous lactate. The results of these are compared with a series of solutions containing no added iron. The stability of the Liver Fraction I is measured by its vitamin content of nicotinic acid, riboflavin and choline.

Experimental

A commercial sample of Liver Fraction I obtained from the Armour Laboratories was assayed for its content of nicotinic acid, riboflavin and choline and compared with the minimum contents of these vitamins as given in the N. F. X. The riboflavin was assayed by the fluorometric method of the N. F. (3), the choline was assayed by the colorimetric method of the N. F. (4) and the nicotinic acid by an adaptation of that of Melnick et al. (5), (6).

TABLE I.

ASSAY RESULTS OF THE ORIGINAL LIVER FRACTION I SAMPLE AS COMPARED WITH THE MINIMUM VALUES OF N. F. X, MG. PER GM.

	Sample	*N. F. Minimum*
Nicotinic Acid	1.147	1.000
Riboflavin	0.2122	0.150
Choline	21.138	10.000

Nicotinic Acid Assay:

An accurately weighed 3-Gm. sample of Liver Fraction I was transferred completely by means of distilled water to a 500 ml. volumetric flask and then made up to volume. Ten ml. of this solution representing approximately 60 micrograms of nicotinic acid was pipetted into a centrifuge tube. Ten ml. of distilled water and 5 ml. of concentrated hydrochloric acid were added and the centrifuge tube was then immersed in a boiling water bath for 30 to 40 minutes with occasional agitation. The sample was then cooled to room temperature and its pH was adjusted to 0.5 to 1.0 by addition of 50 per cent sodium hydroxide solution using methyl violet as an internal indicator.

About 2.5 Gm. of Lloyd's Reagent was added, the suspension was mixed thoroughly for about one minute and then centrifuged. The

supernatant liquid was discarded and the residue was washed twice with 0.2 N sulfuric acid, being certain to break up the residue each time before again centrifuging and discarding the wash liquid.

To the washed residue, 10 ml. of 0.5 N sodium hydroxide solution was added and the mixture was mixed thoroughly for about one minute. Water was then added to bring the contents to a 15 ml. mark on the tube. It was again mixed thoroughly and then centrifuged.

The supernatant liquid was drained off into a clean, dry test tube containing 0.7 Gm. of powdered lead nitrate. After thorough mixing, the suspension was centrifuged and the decolorized, supernatant liquid was transferred to another clean, dry test tube.

One drop of phenolphthalein solution was added followed by solid potassium phosphate until the color of the solution became slightly pink. Then 20 per cent phosphoric acid was added dropwise from a capillary tube until the solution was neutral to nitrazine paper. The suspension was then centrifuged for the last time and the supernatant liquid transferred to another clean, dry test tube having a 15 ml. mark. The liquid was made up to this mark with distilled water and mixed thoroughly.

This solution is the extract for use in the photometric determination of nicotinic acid using a Pfaltz and Bauer Photoelectric Colorimeter with filter at 420 millimicrons.

The following special reagents were used in preparing the colorimetric tubes for readings.

Alcoholic Buffer Solution (equivalent to extracting fluid):

Distilled Water	1960 ml.
Sodium Hydroxide Solution, 15 per cent	30 ml.
Phosphoric Acid, 85 per cent	8 ml.
Absolute Ethyl Alcohol	333 ml.

Cyanogen Bromide Solution:

Reagent Cyanogen Bromide	5 Gm.
Distilled Water	50 ml.

Standard Nicotinic Acid Solution:

U. S. P. Reference Nicotinic Acid	6.825 mg.
Absolute Alcohol, sufficient quantity to make 1000 ml.	

CONTENTS OF COLORIMETRIC TUBES FOR TRANSMISSION DETERMINATION.

Tube	Extract	Buffer Solution	Cyanogen Bromide Solution	Distilled Water	St. Nicotinic Acid Sol.	Anilene Reagent*
1	3 ml.	7 ml.		12 ml.		
2	3 ml.		6 ml.	12 ml.		1 ml.
3	3 ml.		6 ml.	10 ml.	2 ml.	1 ml.
A (Sample Blank)		7 ml.		15 ml.		
B (Reagent Blank)			6 ml.	15 ml.		1 ml.

* This was added 10 minutes after mixing other ingredients in the table.

The transmission readings of tubes (1, 2, 3) were made about five minutes after all the ingredients had been mixed, the time when the readings are at their peak. Before reading tube (1) the colorimeter was set at 100 per cent transmission using tube A. Before reading tubes (2) and (3) the colorimeter was adjusted to 100 per cent transmission using tube B.

The per cent transmission (T) readings of the photometric colorimeter were converted into photometer density (L) by means of the formula:

$$L = 2 - \log T$$

Then by the following calculations, the data for determining the amount of nicotinic acid in the sample could be obtained

L_2 minus L_1 equals L_u Photometric Density due to ml. extract

L_3 minus $L_2 = L_{inc}$ Photometric Density due to 13.65 micrograms of nicotinic acid increment

$$\frac{L_u \times .01365}{L_{inc}} = \text{mg. nicotinic acid in 3 ml. extract}$$

$$\frac{\text{mg. nicotinic acid in 3 ml. extract} \times 1 \text{ Gm. sample}}{\text{Gm. sample represented in 3 ml. extract}} = \text{mg. nicotinic acid per Gm. of sample.}$$

Riboflavin Assay:

In preparing the sample for assay, an accurately weighed portion of about 10 Gm. of Liver Fraction I was dissolved in 10 ml. of distilled water. Its pH was adjusted to 5.5 and its volume made up to about 20 ml. with more distilled water. This solution was autoclaved at 121° C., cooled and diluted in a volumetric flask to 50 ml. A 5 ml. portion of this solution was pipetted into a 1000 ml. volumetric flask and made up to volume with distilled water. 50 ml. of this solution was diluted to 100 ml. in another volumetric flask and this solution constituted the test solution for the riboflavin assay (estimated to contain about 0.1 microgram of riboflavin per ml.) The subsequent assay followed the fluorometric method of the N. F. (3) using a Pfaltz and Bauer fluorophotometer.

Choline Assay:

The choline content was determined by the official N. F. (4) method using a Bausch and Lomb Photo-Electric Colorimeter.

Table I shows that the Liver Fraction I used in this study met the N. F. requirements for the vitamins in every detail.

To simulate commercial liquid preparations containing Liver Fraction I, twenty-seven solutions of 30 ml. each were prepared, nine without added iron, nine with added ferrous gluconate U. S. P. and nine others with added ferrous lactate. In each series the pH was adjusted to range between 3.0 and 7.0. These solutions were assayed for vitamin content of nicotinic acid riboflavin and choline as a measure of the stability of the Liver Fraction I contained in each formula under the specific pH condition at the time of preparation and after an aging period. As part of the vehicle for these solutions, the following buffered solution was used:

Citric Acid	21.0 Gm.
Sodium Hydroxide	8.0 Gm.
Distilled water qs	750.0 ml.

To this was added the following preservative solution, after standing overnight:

Methyl paraben	1.0 Gm.
Propylparaben	0.4 Gm.
Distilled water, boiling qs	250.0 ml.
To make	1000.0 ml.

The first series of nine solutions containing no added iron had the following composition:

Liver Fraction I	3.00 Gm.
Sucrose	7.50 Gm.
Glycerin	2.16 ml.
Buffer solution qs	20.00 ml.
Distilled water qs	25.00 ml.

Nine such solutions were adjusted in order of pH ranging from 3.0 to 7.0 in 0.5 intervals
Distilled water added qs to 30.00 ml.

The second series of nine solutions containing added ferrous gluconate U. S. P. had the following composition:

Liver Fraction I	3.00 Gm.
Sucrose	7.50 Gm.
Glycerin	2.16 ml.
Buffer Solution containing 100 mg. Iron* qs	20.00 ml.
Distilled water qs	25.00 ml.

Nine such solutions were adjusted in order of pH ranging from 3.0 to 7.0 in 0.5 intervals
Distilled water added qs to 30.00 ml.

* Iron from Ferrous Gluconate U. S. P. (assaying 11.5 per cent iron).

The third series of nine solutions containing added ferrous lactate had the same composition and method of adjustment of pH as the second series except that the Buffer Solution containing 100 mg. iron qs 20.00 ml. contained the iron in the form of Ferrous Lactate (assaying 19.3 per cent iron).

The actual technique used in preparing these solutions is given in the publication by Wilken, Lofgren and Burlage (2). Following assays for nicotinic acid, riboflavin and choline immediately after preparation of their solutions, the three series of twenty-seven preparations were aged in an incubator at 37° C. thus permitting some acceleration in reaction over that encountered in an average pharmacy. All the samples were protected from light and only one sample had to be rejected due to mold growth.

The aging period for nicotinic acid varied from 179 to 224 days, for riboflavin from 150 to 195 days, and for choline from 159 to 204 days. These variations occurred due to the varying time required for performing the assays.

After aging, all the samples were assayed and compared with the original assay of the same preparations, expressing the results as per cent activity after aging with the original assays of preparations being considered 100 per cent.

These assays were performed in the same manner as with Liver Fraction I except that different dilutions were made to provide concentrations of vitamins suitable for the assay.

Discussion

Figure I represents the retained potency of vitamins in Liver Fraction I made into solutions buffered at various pH without the addition of an iron compound and aged an average of about 200 days. Nicotinic acid showed two peaks of maximal activity, one peak of about 92% at a pH in the neighborhood of 5 and the second peak of about 99% at a pH of about 6.5. The riboflavin also showed two peaks of maximal activity, the first peak of about 97% in the neighborhood of pH 3.8 and the second peak of 92% at about pH 6.1. The choline had only one peak of about 100% in the neighborhood of pH 5.0 and gradually tapered off as the pH increased. These curves compare fairly well with those of Wilken, Lofgren and Burlage (2), in which the assay of the nicotinic acid and riboflavin were by the microbiological method in contrast to the present assays by photometric methods.

The optimum pH for the Liver Fraction I in the solution without added iron appeared to be between pH 5 and 6. Adjusting a sample of Liver Fraction I solution in this range and aging from 150 to 225 days will give a retained potency of nicotinic acid of from 86%-92%, of riboflavin from 83%-91%, and of choline from 94%-99% as determined by photometric methods of assay. However, since other B vitamins are no doubt present to some extent in Liver Fraction I and are of an acidic nature, the lower pH would be preferred. In the neighborhood of pH 3.5 or 4.0 where any thiamine in the Liver Fraction I would be most stable, the choline would only be present to the extent of about 35%.

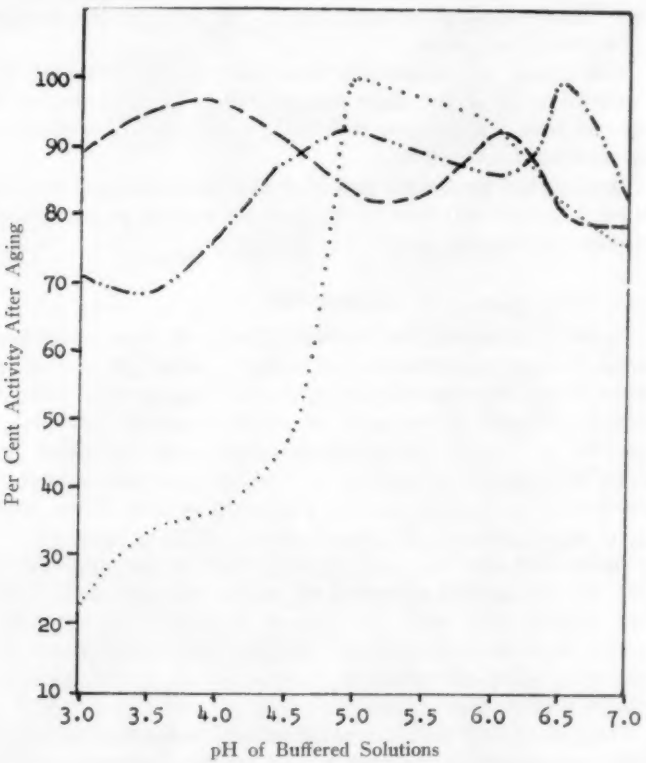

FIGURE 1

THE STABILITY OF LIVER FRACTION I VITAMINS IN SOLUTION WITHOUT THE ADDITION OF AN IRON COMPOUND

Figure 2 represents the retained potency of the vitamins in Liver Fraction I made into solutions buffered at various pH with the addition of iron in the form of ferrous gluconate U. S. P. and aged an average of about 200 days. Nicotinic acid showed three peaks of maximal activity, the first peak of 87% at a pH in the neighborhood of 3.5, the second peak of 83% in the neighborhood of pH 4.8 and the third peak of 91% in the neighborhood of pH 6.3. The riboflavin showed a major peak of 91% at pH 5.5 to 6.0 with a minor peak of about 88% between pH 3.0 and 3.5. Choline had a major peak of 95% at pH 5.0 to 5.3.

The optimum pH for the Liver Fraction I in solutions with added ferrous gluconate appeared to be between pH 5 and 5.5. Adjusting a sample of this solution within this range and aging from 150 to 225 days will give a retained potency of nicotinic acid of from 79%-82%, of riboflavin from 82%-90% and of choline from 92%-95% as determined by the photometric method. In order to attempt to conserve other acidic B vitamins, a pH of 3.5 for the solution might be considered optimum. However, at this pH the retained potency of nicotinic acid was 87%, of riboflavin 88% and of choline 78%.

Figure 3 represents the retained potency of the vitamins in Liver Fraction I solutions buffered to various pH and with iron added in the form of ferrous lactate and aged an average of about 200 days. In this series of solutions, nicotinic acid showed three peaks of maximal activity, the first peak of about 92% at pH 3.5, the second peak of 93% at pH 4.8 and the third peak of practically 100% between pH 6.5 and 6.8. Riboflavin showed no marked peaks of maximal activity but was most stable in the samples at pH 3 and pH 7. At pH 3.0, the activity for riboflavin was 78% and at pH 7.0, it was 77%. Choline showed a peak of activity in the region of pH 4.0 to 4.3 but its maximal activity was from pH 6 to 7. At its peak at pH 4.0 to 4.3 its activity was about 45%, whereas between pH 6 to 7 its activity rose gradually from 80% to 86%.

The optimum pH for the Liver Fraction I solution with added ferrous lactate appears to be at pH 6.75. Adjusting a solution to this pH and aging from 150-225 days will give a retained potency of nicotinic acid of 99%, of riboflavin of 76%, and of choline of 81%. No suitable adjustment of the pH of the solution at a lower pH value in order to conserve other acidic B vitamins in the Liver Fraction I was possible since marked decomposition of choline took place below a pH of 6.

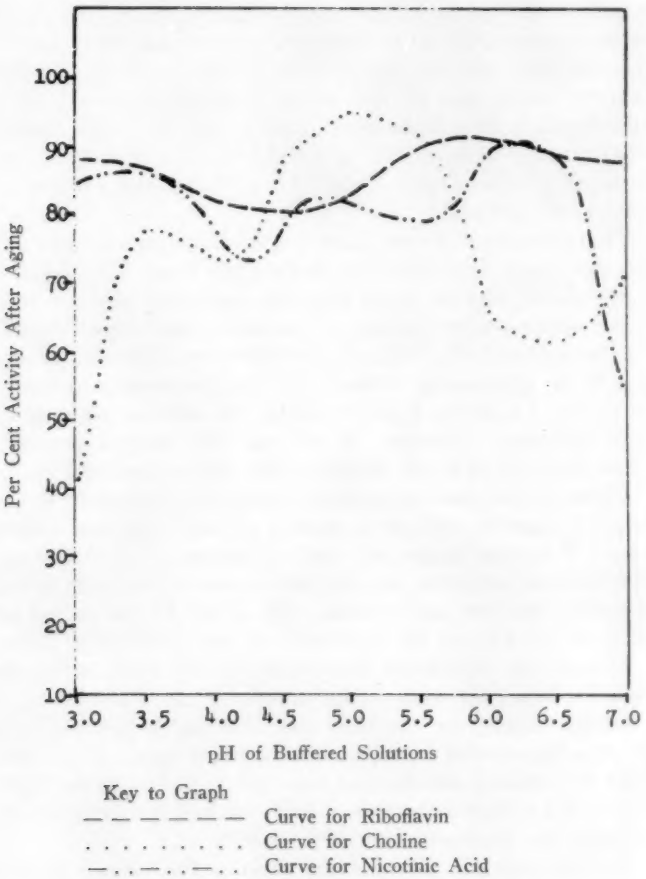

FIGURE 2

THE STABILITY OF LIVER FRACTION I VITAMINS IN SOLUTION CONTAINING FERROUS GLUCONATE

As found in previous work (*2*), the addition of iron compounds to solutions of Liver Fraction I has varying effects on the stability of the vitamins present, effects which differ dependent on the form of iron used. In general, solutions with added ferrous gluconate do not differ markedly from similar solutions without added iron at most pH levels. The riboflavin in retained potency values for the solutions containing ferrous gluconate ranged from 80% to 96% with the peak at about pH 5.75. Solutions with added ferrous lactate gave a considerably different picture, with riboflavin retained potency levels considerably less than in solutions without added iron. Its riboflavin retained potency values ranged from 61% to 80%, with the peaks at pH of 3.0 and pH 7.0. Between these pH values, the retained potency was less with the lowest level at pH 5.25.

Solutions with added ferrous gluconate at many pH levels appeared to decrease the nicotinic acid retained potency level over those levels in solutions without added iron. However, at the pH levels between 3.0 and 4.0, the ferrous gluconate solutions had greater nicotinic acid retained potency levels than in the corresponding solutions without added iron. Aside from this region of pH level 3.0 to 4.0, the curves were somewhat similar, but generally the ferrous gluconate solutions showed less retained potency. With ferrous lactate present in the solutions, the nicotinic acid potency rose to 92% or above three times i.e. at pH 3.5, 4.5 and 6.5. These values equaled or were better than those of the solution without added iron. One marked drop in nicotinic acid retained potency to 65% occurred with the ferrous lactate solution at pH 5.5. The solution without added iron at this pH level exhibited a nicotinic acid retained potency of 89%.

The effect of the iron salts on the choline stability has followed the pattern as established in the solution without added iron i.e. with poor stability below a pH of 5 or 6 but with quite good stability above this pH level. Solutions with added ferrous gluconate has shown a somewhat different pattern in that it demonstrated a choline retained potency at pH 3.5 of 78% and rose to a peak at pH 5 of 95% then dropping gradually at higher pH levels.

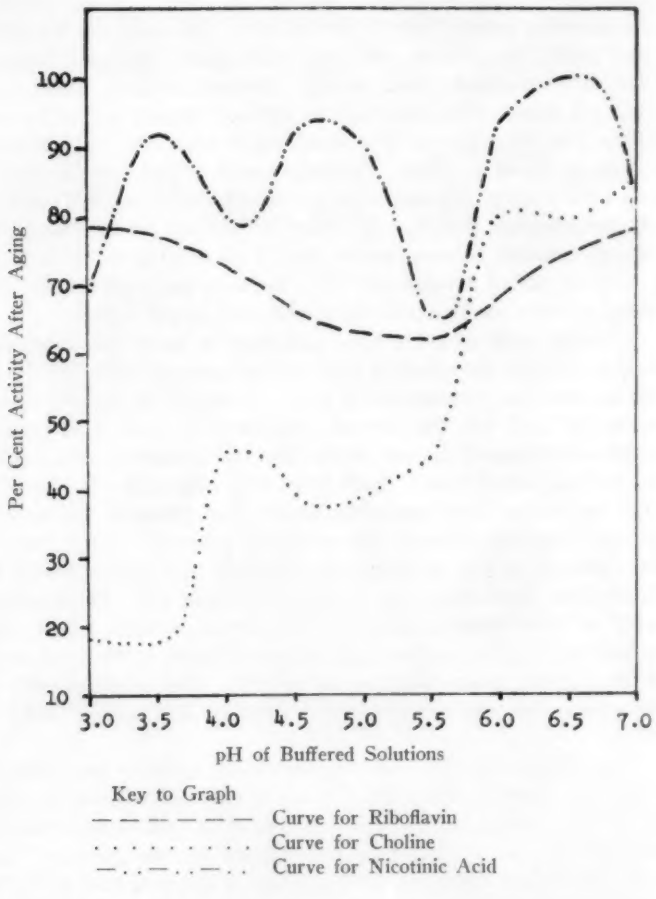

FIGURE 3

THE STABILITY OF LIVER FRACTION I VITAMINS IN SOLUTION CONTAINING FERROUS LACTATE

Conclusions

1. The pH for optimum stability of Liver Fraction I in solution without the addition of an iron compound is between 5.0 and 6.0. This confirms previous results obtained by microbiological assay.

2. The pH for optimum stability of Liver Fraction I in solution with ferrous gluconate U. S. P. is between 5.0 and 5.75.

3. The pH for optimum stability of Liver Fraction I in solution with ferrous lactate is between 6.25 and 6.75.

4. All these assays were conducted by photometric methods.

REFERENCES

(1) "The National Formulary", J. B. Lippincott Company, Philadelphia, Pa., 10th ed., 1955, p. 333.

(2) Wilken, L. O. Jr., Lofgren, F. V. and Burlage, H. M., *Drug Standards, 23,* 74-79 (1955).

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(4) *Ibid.,* pp. 654-55.

(5) Melnick, D., Oser, B. L. and Siegel, L., *Ind. Eng. Chem., Anal. Ed. 13,* 879 (1941).

(6) Melnick, D., *Cereal Chem. 19,* 553 (1942).

TESTING FOR STERILITY OF CORN OIL

Part II

THE USE OF FILTERS AND FILTRATION PROCEDURES *

Kenneth E. Avis ** and Louis Gershenfeld ***

Introduction

AS indicated in the Preface to Part I (1) of this series of studies, bacteria-excluding filters have been used to remove microorganisms from oils. A method has been developed by others (2, 3, 4) whereby microorganisms were removed from an oil by passing the oil through an asbestos pad filter. After rinsing the filter with petroleum ether, it was transferred to culture medium and the entire pad incubated. This method was recommended as a sterility testing procedure for oils.

The following presentation will report the results of an investigation of other filters and filtration techniques. Only corn oil has been used in the following study because of the limitations of time. It is recognized that other oils might have somewhat different effects. Likewise, the scope of the over-all problem being so extensive, only the spores of *Bacillus cereus* ATCC No. 7064 were used in this portion of the study. Other organisms may have displayed somewhat different characteristics if employed with the methods mentioned here.

The dispersion of the spores of *B. cereus* in the corn oil and in saline was performed in the manner described in Part I of this series.

Since bacteria-excluding unglazed porcelain and diatomaceous earth filter candles and asbestos filter pads retain microorganisms during vacuum filtration when present in an oil passed through them, it seemed possible that microorganisms could be removed subsequently from the filters by reversing the flow of liquid. This would be par-

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ticularly useful could it be accomplished using culture media for the reverse flush of the filters. Accordingly, this possibility was investigated.

The effectiveness of the removal of spores by Millipore Filters [a] from corn oil suspensions and of their recovery by the Millipore incubation technique was also investigated.

Filtration Method Involving Reverse Flushing of Filters

Three types of filters were used in the investigation of the effectiveness of the reverse flushing of spores from the filters after an oil sample contaminated with spores had been filtered by vacuum. The unglazed porcelain filters were Selas candles [b] of 01, 015, and 02 porosities. They were 5/8" in diameter and 2" or 4" in length. The diatomaceous earth filters were Mandler candles [c] of 6 to 18 p.s.i. bubbling pressure test. They were 5/8" in diameter and 2" in length. A few trials also were made using a Horm 100 ml. laboratory filter unit [d] provided with a stainless steel screen to support the asbestos pad on both sides. The asbestos pads used were of bacteria-excluding porosity.

All of the filters employed were wrapped in paper and sterilized by autoclaving for at least 20 minutes at 121° C., the open end of the mantle having been previously plugged with nonabsorbent cotton. If time permitted, they were dried at room temperature before use. There was no apparent difference in the effectiveness of the filters whether they were used moist or dry.

Measured volumes of corn oil, ranging in volume from 20 to 100 ml. and contaminated with spores of *B. cereus*, were filtered through the filters by means of vacuum. The filtrate was collected in sterile filter flasks. The vacuum was then broken by allowing air to enter the system through a sterile air filter.

After the filtration of the corn oil sample, sterile solutions of detergents in saline were passed through the filters, followed by sterile saline to remove the detergent. The detergent solution employed was either a mixture of 0.1 to 0.2 per cent Tween 80[e] and 0.15 to 0.3 per

[a] Millipore Filter Corp., Watertown, Mass.

[b] Supplied by Selas Corp. of America, Dresher, Pa.

[c] Supplied by Allen Filter Co., Toledo, O.

[d] Supplied by F. R. Horman & Co., Inc., Newark, N. J.

cent G-2800[e] (T-G), or a solution of 0.1 to 0.3 per cent Triton X-100[f] (TrX) in saline. The combined filtrates were usually tested for sterility by transferring 5 one ml. inocula to tubes containing 10 ml. of trypticase soy broth (TSB) or Fluid Thioglycollate Medium (FTM). These served as additional controls.

After filtration was completed, the filter unit was aseptically removed from the flask and secured by means of a clamp to a ring stand. A pressure bottle containing TSB was then attached aseptically to the filter nipple (See Figure 1). Pressure was then increased in the bottle from a tank of nitrogen through a sterile air filter. Depending on the porosity of the filter, a pressure of 4 to 15 p.s.i. was required to force the TSB through the filter in the reverse direction from that of filtration. When from 20 to 50 ml. of TSB (sufficient to cover the candle) had accumulated in the mantle, the hose leading from the pressure bottle was pinched closed with a clamp, carefully slipped from the nipple attachment, and then the TSB in the mantle was aseptically transferred to a sterile culture tube for incubation. The total volume of TSB was incubated in test tubes having a diameter of 25 mm. and a length of 150 or 200 mm.

The pressure bottle containing the sterile TSB was prepared in the following manner. A clean, standard 500 or 250 ml. parenteral solution bottle was provided with a two-hole stopper and aluminum seal. Two "L" shaped glass tubes were inserted through the holes in the stopper. The short one served as the nitrogen inlet tube, the other extended to the bottom of the bottle and served as the TSB outlet tube. During autoclaving, however, the latter was left above the surface of the TSB. The TSB was drawn into the bottle by means of vacuum. The outside ends of both tubes were plugged with nonabsorbent cotton. The tubes, including the top of the bottle, were wrapped carefully with nylon cloth and paper. The unit was then sterilized by autoclaving.

Prior to use, the paper was carefully removed from the supply tubes extending above the pressure bottle. Before the nylon cloth was removed, the long TSB outlet tube was pushed to the bottom of the bottle. The nylon cloth was removed, the cotton was removed from the TSB outlet tube, and a length of sterile rubber tubing was

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[e] Supplied by Atlas Powder Co., Wilmington, Del.

[f] Supplied by Rohm and Haas, Philadelphia, Pa.

slipped onto the protruding end of the TSB outlet tube. The tubes were then attached as shown in Figure 1.

Before cleaning, the candle filters were sterilized by autoclaving. They were then reverse flushed with distilled water. Attempts were made to remove the corn oil from the candles by soaking them in detergents or in carbon tetrachloride or a mixture of carbon tetrachloride and petroleum ether. This was not satisfactory. Superior results were obtained, however, when Selas candles were soaked for about 24 hours in a solution of about 0.5 N sodium hydroxide, followed by a thorough rinsing with distilled water. After cleaning, the candle filter units were wrapped and sterilized for re-use, as mentioned previously.

Findings From the Reverse Flushing of Filters

The reverse flushing filtration method for the recovery of the spores of *B. cereus* from corn oil showed promise early in the work.

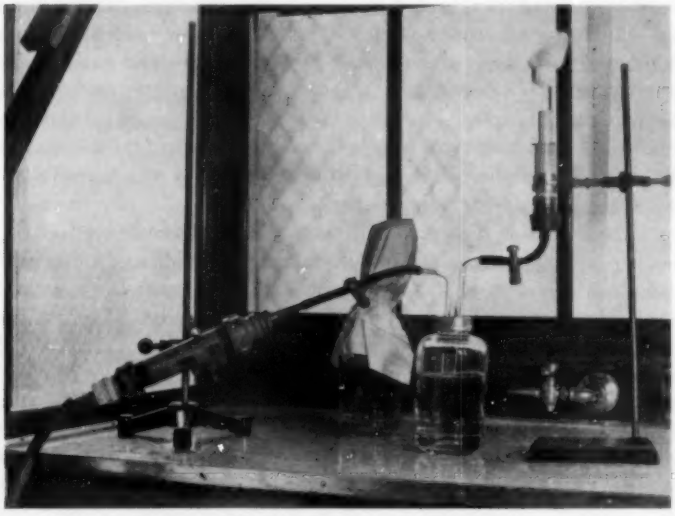

FIGURE 1.

APPARATUS FOR REVERSE FLUSHING CANDLES WITH CULTURE MEDIUM.

However, most of the tests were performed with oil samples containing a large number, theoretically, of spores. The theoretical number of spores was based upon the number of spores in the volume of the original saline suspension added to the test tubes and then dried. These dried spores were then dispersed in the corn oil used for the filtration.

As the number of spores was reduced in the volume of oil filtered, growth was not consistently obtained in the TSB flushed in reverse through the filters following filtration of the corn oil sample. As may be seen from the data recorded in Table I, growth was obtained in the TSB reverse flushed from Selas candles in three out of twelve filtrations when as few as 1.5 to 5 spores were present in the volume of oil filtered. On the other hand, at spore densities of more than 5,000 in the total volume of oil filtered, most of the reverse flushed TSB showed growth, but a few did not. The few negative growth tests at these very high spore densities might be considered to be chance findings were it not for the fact that 38 of 53 TSB samples showed no growth when the spore density in the total volume of oil filtered was between 1000 and 5000. It may also be noted from the data recorded in Table I that the Selas candles having the smaller pore diameter (Selas 02) did not show evidence of being significantly more effective in spore recovery than those having larger pore diameters (Selas 01 and 015). The sterility tests performed on the combined filtrates (corn oil, detergent, and saline) from these candles showed no growth except in a few cases from the filtrate from the Selas 01 candle, which is not designated by the manufacturer as having bacteria-excluding porosity.

The Mandler diatomaceous earth candles were not effective in recovering spores from corn oil filtrations, as evidenced by negative findings for growth in the reverse flushed TSB, when approximately 5000 spores were present in the volume of oil filtered. The difficulty of cleaning these candles, their increased fragility when soaked with corn oil, and the possibility of their becoming less effective from continued use with oils, as reported previously (5), are contraindications for the use of these filters. Table I also provides data from the few trials with the Horm asbestos pad filter. This data indicated that the Horm asbestos pads were not effective in the recovery of the spores of *B. cereus* from corn oil by the method employed.

TABLE 1

RECOVERY OF B. CEREUS SPORES FROM CORN OIL BY A FILTRATION METHOD INVOLVING REVERSE FLUSHING OF FILTERS

Number of Spores[a] Per Volume of Corn Oil Filtered[b]	Selas 01 Candles		Selas 015 Candles		Selas 02 Candles		Mandler Candles		Horm Pads	
	G[c]	NG[d]	G	NG	G	NG	G	NG	G	NG
1.5-5	3	6	0	1	0	2	—[e]	—	—	—
10-25	—	—	1	7	1	0	—	—	—	—
50-120	0	2	3	1	1	0	—	—	0	1
150-250	0	5	3	7	0	1	—	—	—	—
400-600	0	4	8	12	—	—	0	1	0	1
1,000-2,000	0	2	2	9	3	7	—	—	—	—
2,500-5,000	3	6	5	12	2	2	2	4	0	1
15,000-40,000	2	0	5	0	4	0	—	—	1	0
60,000-100,000	2	0	15	1	3	0	5	0	—	—
above 100,000	2	0	3	1	—	—	—	—	—	—
Total Tests	12	25	45	51	14	12	7	5	1	3

[a] Based upon the count of the number of *B. cereus* spores in the suspension dried in test tubes. These were dispersed in the corn oil samples.

[b] Volume of corn oil filtered varied from about 20 to 100 ml.

[c] G = Growth.

[d] NG = No Growth.

[e] — = No Data.

A total of 175 filtration tests were conducted with all types of candles and with the asbestos pad filter. While the results showed that spores of *B. cereus* could be recovered from these filters by means of reverse flushing the filter with TSB following the filtration of a contaminated oil sample, recovery of the spores was not consistent. The complexity of the method would also provide the ever lurking danger of inadvertent manipulative contamination, with resulting false positive tests. To be practical as a test method, such an involved procedure should be strikingly superior to existing test methods. This did not prove to be the case.

Millipore Filtration

Packs of Millipore filters, interleaved with absorbent pads, were wrapped tightly in paper or secured in open-top Brewer Petri dishes and sterilized by autoclaving. The base support and the funnel of the glass filter unit were also sterilized by autoclaving.

At the time of use, the filter membrane was aseptically transferred to the filter base with sterile forceps, the base having previously been inserted in position in a 500 ml. sterile filter flask. The funnel was then placed in position and clamped tight. The usual inoculum filtered from the spore suspension was 0.1 ml. In order to better distribute the organisms over the surface of the filter, about 3 ml. of sterile vehicle (corn oil or saline) were first placed in the funnel and the 0.1 ml. inoculum was added. When more than one liquid was passed through the filter membrane by vacuum, each was added to the funnel in portions and each portion allowed to pass through before another was added.

While filtration was proceeding, an absorbent disc was aseptically transferred to the bottom of a sterile Petri dish having a Brewer solid aluminum top and an absorbent liner. Enough sterile TSB was added to saturate the absorbent disc. After filtration was completed, the filter membrane was transferred to the absorbent disc by means of a sterile spatula, making sure that all air bubbles were out from under the filter so that complete contact could be made with the medium in the absorbent disc. The covered dish was inverted and sterile water poured into the space between the aluminum lid and the glass bottom. The liner absorbed this water and a humid atmosphere was provided during the incubation of the filter membrane, the dish being in the inverted position.

The filters were examined with a 10x hand lens for the appearance of colonies after incubation at 30 to 32° C. Optimum growth usually appeared in about 40 hours and, sometimes, later. The colonies of *B. cereus* were usually white or slightly cream-colored with a granular texture. They were round and markedly dome-shaped when no surface active agents were present. When surface active agents were present or growth had extended over several days, they were relatively flat, irregular in outline, and spread extensively over the surface of the filter.

The filters were rinsed with several different liquids, before and/or after the corn oil samples were filtered, in attempts to increase the recovery of organisms. Saline, detergent-saline (T-G or TrX),

and petroleum ether were used prior to the oil sample filtration to determine the effect of saturating the filter with another liquid before the oil. The petroleum ether used was the 30 to 60° C. boiling fraction. These same liquids were also used after the oil sample had filtered through so as to wash the oil from the spores retained on the surface of the filter and, thus, permit more of them to grow. Carbon tetrachloride was used in a few trials.

Colony counts from Millipore filtrations were compared with those obtained from pour plates made with inocula from the same spore suspensions, the latter being used as controls. The colony counts obtained on Millipore filters were expressed as percentage of recovery when compared with the control counts on pour plates.

The pour plates were prepared by inoculating duplicate or triplicate tubes of liquid trypticase soy agar (TSA), usually at a temperature of about 45° C., with 0.1 ml. portions from the saline spore suspension and a like set of tubes with 0.1 ml. portions from the corn oil spore suspension used for the Millipore filtrations. These were then shaken thoroughly and poured into sterile Petri dishes. The saline spore suspension was prepared in an identical manner with that of the corn oil suspension except that saline was used as the diluent instead of corn oil. Both the saline and the corn oil suspension were prepared as described in Part I (1).

Findings From Millipore Filtration

The spores of *B. cereus* could be retained on Millipore filters following the passage of a corn oil suspension of the spores through them. The number of spores retained on the filters and observed to develop into colonies under incubation conditions, was found to vary appreciably depending upon the treatment given the filters before and after the oil filtration and whether or not the oil was mixed with Tween 80 prior to filtration.

Table II contains the data from these experiments. The recovery of spores is recorded on the basis of the mean percentage of recovery from the corn oil suspensions on the Millipore filters as compared with the recovery on two TSA pour plates made from an inoculum from a saline suspension of spores prepared at the same time and, two other TSA pour plates made with an inoculum from the same corn oil suspension used for the filtration. The recovery was indicated by the number of typical colonies of *B. cereus* observed to develop following incubation.

TABLE II

RECOVERY OF B. CEREUS SPORES FROM CORN OIL WITH MILLIPORE FILTERS AS COMPARED WITH POUR PLATE [a] RECOVERY FROM UNFILTERED SALINE AND CORN OIL SUSPENSIONS

Method of Treatment[c]	No. of Millipore Filtrations	Mean Percentage of Recovery with Millipore Filters as Compared with: Pour Plates Inoculated with Unfiltered Saline Suspension	Mean Percentage of Recovery with Millipore Filters as Compared with: Pour Plates Inoculated with Unfiltered Corn Oil Suspension
N	5	139	—[b]
P*	13	2.7	13
P	4	0.09	0.25
Q*-1	9	125.5	63
Q*-2	10	12.5	69
Q*-3	15	95.8	568
Q*-4	4	3.5	25.4
Q-5	2	2.7	22
Q-6	1	26.6	200
Q-7	3	9	26.6
R*	2	66	100
S*	2	6.7	20
T	5	64	99
U	2	0	0
V	2	79.8	140
W	7	45	122
X	10	41.3	91
Y	1	46	140
Z	7	4	18

[a] Inoculum shaken with trypticase soy agar (TSA) and poured into Petri dish. Colonies counted after 12, 24, 48, and 72 hours incubation at 30-32° C.

[b] — = No data.

[c] Tween 80 was mixed with corn oil suspension, usually in ratio of 1 part Tween 80 to 10 or more parts corn oil.

[d] *Treatment Methods*

N—saline spore suspension only filtered.

P—corn oil spore suspension only filtered.

Q—filtration order: corn oil suspension, detergent-saline, saline.

Q-1—detergent-saline rinse was 0.1% Tween 80 and 0.15% G-2800 (T-G), less than 50 ml.

Q-2—detergent-saline rinse was 0.1% Triton X-100 (TrX), less than 50 ml.

Q-3—detergent-saline rinse was T-G, 50 to 100 ml.

Q-4—detergent-saline rinse was TrX, 50 to 100 ml.

Q-5—detergent-saline rinse was TrX, less than 50 ml.

Q-6—detergent-saline rinse was T-G, 50 to 100 ml.

Q-7—detergent-saline rinse was T-G, less than 50 ml.

R—filtration order: detergent-saline, corn oil suspension, detergent-saline, saline. Detergent-saline was T-G, 50 to 100 ml.

S—filtration order: saline, corn oil suspension, detergent-saline, saline. Detergent-saline was T-G, 50 to 100 ml.

T—filtration order: corn oil suspension, petroleum ether.

U—filtration order: petroleum ether, corn oil suspension.

V—filtration order: petroleum ether, corn oil suspension, petroleum ether.

W—filtration order: corn oil suspension, petroleum ether, saline.

X—filtration order: corn oil suspension, petroleum ether, detergent-saline, saline.

Y—filtration order: corn oil suspension, carbon tetrachloride, saline.

Z—filtration order: corn oil suspension, carbon tetrachloride, detergent-saline, saline.

The dilutions of the original sample used for these experiments ranged from no dilution to 1:2000. Most of the dilutions, however, were no greater than 1:100. In computing the mean percentage recovery, a few results which were very high were excluded from the computation. In each case, these abnormally high results appeared to be due to the multiplication factor resulting from high dilutions of the original sample.

The percentage recovery of the spores of *B. cereus* with Millipore filters, in practically all treatment categories, compared more favorably with the pour plates inoculated from the corn oil suspension than with the pour plates inoculated from the saline suspension. This fact, evident from the data in Table II, would be expected since colony counts in TSA from corn oil inocula were lower than those from saline inocula. It is well known that spores grow with less frequency from oils than from aqueous liquids.

The methods of treatment, tabulated at the bottom of Table II, involved different sequence for the filtration of the corn oil suspension and various rinsing liquids as well as different volumes of the rinsing liquids.

From the data listed in Table II, the following also may be noted. The recovery of spores from saline suspensions (Treatment N) appeared to be slightly more effective when performed by Millipore filtration than by pour plates. However, the recovery of spores from corn oil suspensions (Treatments P) was much less effective when performed by Millipore filtration without supplemental treatment of the filter, than by pour plate. The incorporation of 1 part of sterile Tween 80 with 10 or more parts of the corn oil suspension before filtration (treatments marked with asterisk) increased the recovery of spores from the oil. Rinsing the Millipore filter with detergent-saline and then saline, following corn oil filtration (Treatments Q), increased the recovery of spores as compared with no rinsing (Treatments P). Triton X-100, in a concentration of 0.1 per cent, was less effective as a rinsing agent (Treatments Q-2, -4, -5) than was a mixture of 0.1 per cent Tween 80 and 0.15 per cent G-2800 (Treatments Q-1, -3, -6, -7). The volume of detergent-saline rinse used following the corn oil filtration had a striking effect, when the detergent was the T-G mixture. When the volume of the detergent-saline rinse was 50 to 100 ml. (Treatments Q-3, -6), the recovery was much greater than when the volume was less than 50 ml. (Treatments Q-1, -7).

The most effective recovery of spores was obtained when the corn oil suspension was mixed first with Tween 80 and then the Millipore filter was rinsed with more than 50 ml. of the T-G mixture in saline (Treatment Q*-3). Almost as many spores were recovered on the Millipore filters from the corn oil suspension (95.8 per cent) as were recovered from the saline suspension inocula in TSA. More than five times as many spores were recovered on the Millipore filters from

the corn oil suspension than were recovered from the corn oil suspension inocula in TSA.

Soaking the Millipore filters with detergent-saline, saline or petroleum ether prior to the filtration of the corn oil suspension did not improve the recovery, with the possible exception of the two procedures in which petroleum ether was used prior to and also after the corn oil filtration (Treatment V). In each of the procedures in which either petroleum ether or carbon tetrachloride was used to rinse the filters following the corn oil filtration and a final saline rinse was employed (Treatments W, Y), a greater recovery of spores was obtained than from corn oil inocula in TSA.

Summary and Conclusions

Spores of *B. cereus* were recovered in TSB which was forced in the reverse direction through unglazed porcelain and diatomaceous earth filter candles and Horm asbestos pad filters. The filters had been used previously to filter, by vacuum, a sample of corn oil inoculated with the spores. However, recovery was not consistent. No growth was obtained in 96 of 175 tests (55 per cent). Even when the theoretical number of spores in the volume of oil filtered was 2500 or more, 32 per cent of the tests were negative for growth. Although this method at first appeared promising, particularly because of the large volume of oil inoculum that could be employed relative to culture tube techniques, the results indicate that the method was not superior to other techniques.

The recovery of spores from corn oil suspensions with Millipore filters in a total of 104 tests was compared with the recovery of spores from inocula in TSA positive control pour plates from saline spore suspensions and from the same corn oil spore suspension used for the filtration. The filters were rinsed after, and sometimes before, the filtration of the corn oil suspension with one or more liquids, including, detergent-saline, saline, petroleum ether, and carbon tetrachloride. The most effective recovery of spores was obtained when, after the filtration of the corn oil suspension, the filter was rinsed with 50 to 100 ml. of a detergent-saline mixture of 0.1 per cent Tween 80 and 0.15 per cent G-2800 and then about 50 ml. of saline. Prior to filtration, the corn oil suspension had been mixed with approximately 1 part sterile Tween 80 to 20 parts corn oil. The recovery of spores with the Millipore filter using this technique was almost equal to the recovery

from a saline suspension in TSA pour plates (95.8 per cent) and about 5 times greater (568 per cent) than the recovery from the corn oil suspension in TSA pour plates. These results would suggest that this method of recovery of spores from corn oil suspensions warrants further investigation.

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DRUG INFORMATION SOURCES *

(Mexico, Colombia and Argentina)

Vademecum Internacional de Especialidades Farmacéuticas y Biológicas. Ridgewood, N. J., U. S. A., J. Morgan Jones Publications, Ltd.

Four of these handbooks, all in Spanish, are published for the Latin American countries. They are based on information supplied by pharmaceutical manufacturers. Each contains a list of manufacturers with selected products and detailed information about composition, action, indications, administration, dosage and forms and sizes supplied; a therapeutic and pharmacologic index; and a list of laboratories with all products sold but no other information than name of product. An alphabetic list of products is sometimes included.

The four Latin American editions are named according to the area covered: Cubard for Cuba and Dominican Republic; Meca for Mexico and Central America; Covper for Colombia, Ecuador, Venezuela and Peru; and Abcupar for Argentina, Bolivia, Chile, Uruguay and Paraguay. Information for these editions is supplied by pharmaceutical laboratories in the United States, Canada, England, Holland, Germany, Switzerland, Italy, France, and, depending on the area of circulation, Cuba, Mexico City, Mexico, Colombia, Venezuela, Ecuador and Argentina. Publisher's address: 75 North Maple Ave., Ridgewood, N. J., U. S. A. and 6306 Park Ave., Montreal 8, Quebec.

MEXICO

Diccionario de Especialidades Farmaceuticas, ed. by I. Landero and E. Rosenstein. 7th ed. Mexico D. F., P. L. M., 1954. 1148 pp.

A handbook of information about medical specialties sold in Mexico. Drugs are entered alphabetically by proprietary name and

* A World List; compiled by the Pharmaceutical Section, Science-Technology Division, Special Libraries Association.

entries include brief description, composition in detail, dosage forms, method of administration, list of indications, precautions where applicable, forms supplied, manufacturer and address. Products included are prescription items only. An index of drugs by pharmacologic action or therapeutic indication and a general alphabetic index are also included. Publisher's address: Desp. 304, Balderas 36, Mexico D. F.

Productos Quimicos y Farmaceuticos, by Prof. Francisco Giral. Mexico D. F., Editorial Atlante, 1946. 3 vols. (2200 pp.) $16.00.

An encyclopedia of basic chemistry, pharmacology and applications of inorganic and organic chemical and medicinal substances. The text includes 1131 products, with background notes on the most important ones and an extensive general introduction to each of the principal groups. Available from: Editorial Grijalbo, Avenida Granjas 82, Mexico 16, D. F.

La Plantas Medicinales de Mexico, by Prof. Maximino Martinez. 3d ed. Mexico D. F., Ediciones Botas, 1944. 630 pp.

Monographs are entered under Mexican name of plant. They report botanic name and other common names, habitat, description and properties, useful parts, chemical composition, pharmacologic action, therapeutic properties and dosage. Folk usage is given if no medical application has been established. Monographs are full and descriptive, with illustrations and references to cited sources. All plant names are included in the general alphabetic index.

COLOMBIA

Colombia. Servicio de Sanidad Militar. **Lista Clasificada de Drogas.** Bogota, Antares, 1954. 64 pp. Free.

A handbook of drugs of recognized utility, compiled for the use of health officials. In pages 1-25 drugs are entered in broad classes according to therapeutic action, as antihistamines, analgetics, antiparasitics, etc. Drugs are entered by common or generic name and available dosage forms are given for each. Proprietary names are

included for specialties. A chart of antibiotic activities and a chapter on cortisone and hydrocortisone complete the text. The general alphabetic index is helpful, but by no means inclusive.

Colombia. Caja Nacional de Prevision. **Farmacia; Memento Medico.** Bogota, 1954. 91 pp.

List of drugs available in Colombia, grouped by indications. Drugs are listed by generic or common name and dosage forms are given.

ARGENTINA

Manual de Especialidades Medicinales, by Aaron Kaplan. 3d ed. Buenos Aires, Lopez & Etchegoyen, 1951. 778 pp.—Supplement. 1953. 139 pp. Bound together, $ argentinos 250.-

Entries are listed alphabetically by proprietary name of drug or combination. Information includes manufacturer, composition, indications, administration, dosage and forms and sizes supplied. A separate alphabetic index lists specialties under indication or common name of drug. There is also a list of manufacturers with their addresses and products. Publisher's address: Junin 863, Buenos Aires.

Guia de Especialidades Medicinales, by Dr. Cesar F. Vallory. Buenos Aires, Editorial Bibliografica Argentina, 1950. 680 pp. $ argentinos 320.-

Part I is an extensive alphabetic list of drug specialties sold in Argentina. For each preparation is given its manufacturer, composition, indications, dosage and package forms and sizes. Part II is a therapeutic index and Part III an index of manufacturers with their addresses and names of their products. Publisher's address: Cangallo 860, Buenos Aires.

Guia Moderna de Terapeutica Clinica, by Dr. Cesar F. Vallory. 3d ed. Buenos Aires, Editorial Bibliografica Argentina, 1949.

Clinical descriptions of diseases and their treatment; official formulas and pharmaceutical specialties, diets and the usual therapeutic agents, streptomycin, penicillin, sulfonamides, vitamins, etc. Publisher's address: Cangallo 860, Buenos Aires.

Interpretacion de Recetas, by Lucas DeFelice. **Suplemento no. 1—** Buenos Aires, "El Ateneo", 1940— .

An important compilation of chemical, pharmaceutical and therapeutic studies of selected official formulas. Chapters are arranged alphabetically according to class of remedy or family of drugs (Antianemia drugs, anthelmintics, antipyretics, bismuth compds., etc.). General discussion covers the clinical picture of diseases and the general action of specific drugs. Monographs on individual drugs give description, chemistry, dosage, formulas for preparations, actions and contraindications. Specialties are frequently listed with their composition and name of manufacturer. Authorities are cited by name, but literature references are not included. The Committee has located only one supplement (Aceites medicinales to Dentifricos) for examination; reference has been located to at least one other volume.

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SELECTED ABSTRACTS

The Effect of the Solvent on Toxicity Tests in Mice. Maffii, G., Semenza, F., and Soncin, E. *J. Pharm. Pharmacol. 9:*105 (1957). The toxicity of the tetracyclines is usually determined by a rapid intravenous injection of 0.5 ml. of a solution containing 2 mg. per ml. of the antibiotic under test into five mice. The method established by the F. D. A. has been almost universally accepted. However, in some cases distilled water is used as the solvent and in others, physiological saline. This difference in the solvent used is due largely to differences introduced in pharmacopoeias.

The authors undertook a series of studies to determine the effect of the vehicle on the toxicity. They found that the hydrochlorides of tetracycline, oxytetracycline, chlortetracycline, and bromtetracycline showed significantly higher LD50 values in terms of mg. per Kg. when physiological saline was used as the solvent as compared with distilled water as the solvent. This finding was obtained not only when mice were used as the test animals but also when rats and guinea pigs were used. The significance of hypotonicity in this matter was shown by further tests in which higher concentrations of tetracycline were used. The per cent mortality was lower when a higher concentration of tetracycline was used, both concentrations employing water as the vehicle. When saline was used as the vehicle in these same two concentrations, the per cent mortality increased with the higher concentration of tetracycline. In further tests using saponins and hydrochloric acid, it was found that the toxicity was significantly higher when distilled water was used as the vehicle as compared with physiological saline.

The authors concluded that the hypotonicity of the solutions unfavorably influenced the resistance of the mice to intravenous injections of the antibiotics. They suggested, therefore, that in all toxicity tests for the tetracyclines, physiological sodium chloride solution should be used as the vehicle rather than distilled water.

The Stabilization of Sulfadiazine Sodium Injection. Hom, F. S., and Autian, J. *Bull. Am. Soc. Hosp. Pharm. 14*:177 (1957). Studies were undertaken to develop a stable formula for sulfadiazine sodium injection. A series of formulas containing either 25 or 50 per cent (w/v) sulfadiazine sodium were prepared, sealed in ampuls and stored at 5° C., at 25° C. in the dark, at 25° C. in direct rays from the sun, and at 60° C. The criteria for determining whether or not a particular formulation was stable was based upon the development and intensity of color and the presence or absence of precipitation. The degree of color change was measured by means of the per cent transmittancy using a spectrophotometer at 500 mμ. Preliminary U. S. P. XV assay procedures showed little difference between a freshly prepared solution and one which had turned to a brownish-red color. It would seem that the U. S. P. assay procedure did not distinguish degradation products from the sulfadiazine molecule or that the substance causing the color in the solutions was present in such an insignificant amount that the assay could not detect the change.

From an evaluation of the results, it was apparent that oxygen was the primary factor in bringing about the deterioration of sulfadiazine sodium. Light also was found to have a catalytic effect. The precipitates which formed in some formulations were not identified.

The authors concluded that the following formula appeared to be satisfactory:

Sulfadiazine Sodium U. S. P.	250 Gm.
Sodium Sulfite, Exsiccated U. S. P.	1 Gm.
(or Sodium Formaldehyde Sulfoxylate)	
Water for Injection, to make	1000 ml.

The following procedure was recommended. Approximately 900 ml. of Water for Injection were heated to boiling in an all-glass apparatus and saturated with nitrogen while cooling to 35° C. The sulfadiazine sodium and antoxidant were added and dissolved and sufficient additional W. F. I. (nitrogen saturated) was added to volume. The solution was then filtered through a sintered glass filter under a blanket of nitrogen and filled into ampuls and sealed under nitrogen. Polyethylene glycol was found to be a desirable adjunct to the formula but was not included since it had not been evaluated pharmacologically for safety with a sulfonamide.

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